CLAIMS

1. A process for manufacture of long circulating non-pegylated liposomes comprising; forming a lipid film by evaporating a solvent from a lipid solution comprising one or more phospholipids, a sterol and a solvent; hydrating the lipid film with an aqueous hydration media to form non pegylated liposomes;
wherein the amount of aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution.

2. The process of claim 1 wherein the amount of aqueous hydration media used is 30 ml for each mmole of phospholipid in the lipid solution.

3. The process of manufacture of non-pegylated liposomes of claim 1 further comprising loading the liposomes with a therapeutic or diagnostic agent.

4. The process of claim 3, wherein the therapeutic agent is an antineoplastic agent.

5. The process of claim 4, wherein the antineoplastic agent is selected from the group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin hydrochloride.

6. The process of claim 5, wherein the antineoplastic agent is Doxorubicin hydrochloride.

7. The process of claim 1, wherein the molar ratio of phospholipid to sterol is from about 1:0.1 - 1:2.

8. The process of claim 7, wherein the wherein the molar ratio of phospholipid to sterol is from about 1:0.7.

9. The process of claim 1, wherein the aqueous hydration media comprises ammonium sulfate and sucrose.

10. The process of claim 9, wherein the concentration of ammonium sulfate in aqueous

hydration media is not less than 125 mmoles/liter.

11. The process of claim 1, wherein the phospholipid has a phase transition temperature of 40°C to 60°C.

12. The process of claim 11, wherein the phospholipid has a minimum of sixteen carbons fatty acid chain.

13. The process of claim 12, wherein the phospholipid is selected from the group consisting of Distearoyl phosphatidylcholine (DSPC), Dipalmitoyl phosphatidylcholine (DPPC), Hydrogenated soya phosphatidylcholine (HSPC) and derivatives of such phospholipids.

14. The process of claim 13, wherein the phospholipid is distearoyl phosphatidylcholine (DSPC) and wherein the sterol is cholesterol.

15. The process of claim 1, wherein the non-pegylated liposomes are successively extruded through series of filters having pore sizes from 0.4μm to 0.05μm for sizing.

16. A liposome manufactured by the process of claim 1.

17. The liposome of claim 16, wherein the phospholipid comprises distearoyl phosphatidylcholine (DSPC) and the sterol comprises cholesterol.

18. The liposome of claim 16, wherein the non-pegylated liposome further comprises a therapeutic or diagnostic agent.

19. The liposome of claim 18, wherein said therapeutic agent comprises an antineoplastic agent.

20. The liposome of claim 19, wherein the antineoplastic agent is selected from the group consisting of Doxorubicin hydrochloride, Daunorubicin hydrochloride, and Epirubicin hydrochloride.

21. The liposome of claim 20, wherein the antineoplastic agent is Doxorubicin hydrochloride.

22. The liposome of claim 16, wherein the average size of liposome is 0.06μm to 0.16μm in diameter.

23. A long circulating non-pegylated liposomal doxorubicin composition for parenteral administration comprising, doxorubicin hydrochloride non-pegylated liposomes, histidine hydrochloride, and sucrose;
wherein the doxorubicin non-pegylated liposomes comprise distearoylphosphatidyl choline, cholesterol, sucrose;
wherein the liposomes have an average size 0.06μm to 0.16μm; and
wherein the non-pegylated doxorubicin liposomes have a circulation time in blood at least 25 times longer than that of ADRIAMYCIN when tested in Swiss albino mice at equivalent doses.

24. The composition of claim 23, wherein the doxorubicin concentration encapsulated in the liposomes is from 1mM to 10mM expressed as doxorubicin hydrochloride.

25. The composition of claim 24, wherein the doxorubicin hydrochloride concentration is from 3mM to 7mM.

26. The composition of claim 25, wherein the doxorubicin hydrochloride concentration is about 3.45mM.

27. The composition of claim 25, wherein the doxorubicin hydrochloride concentration is about 6.9mM.

28. The composition of claim 23, wherein the molar ratio of distearoylphosphatidyl choline to cholesterol is from 1:0.6 to 1:0.8.

29. The composition of claim 28, wherein the molar ratio of distearoylphosphatidyl

choline to cholesterol is about 1:0.7.

30. The composition of claim 23, wherein the molar ratio of doxorubicin hydrochloride to distearoylphosphatidyl choline is from 1:2 to 1:15.

31. The composition of claim 30, wherein the molar ratio of doxorubicin hydrochloride to distearoylphosphatidyl choline is about 1:3.5.

32. The composition of claim 23, wherein the sucrose concentration is from 0.1M to 0.5M.

33. The composition of claim 32, wherein the sucrose concentration is about 0.29 M.

34. The composition of claim 23, wherein the concentration of histidine hydrochloride is from 1 to 100mM.

35. The composition of claim 34, wherein the concentration of histidine hydrochloride is from 8 to 12mM.

36. The composition of claim 35, wherein the concentration of histidine hydrochloride is about 10 mM.

37. The composition of claim 23, wherein the average size of the liposomes is from 0.08μm to 0.12μm.

38. The composition of claim 23, wherein the doxorubicin hydrochloride is present at 2 mg/ml; and
wherein the molar ratio of doxorubicin to DSPC is 1:3.5; and
wherein the ratio of DSPC to cholesterol is 1:0.7.

39. The composition of claim 23, wherein the doxorubicin hydrochloride is present at 4 mg/ml; and
wherein the molar ratio of doxorubicin to DSPC is 1:3.5; and

wherein the ratio of DSPC to cholesterol is 1:0.7.

40. The composition of claim 23, wherein circulation time (t½) in blood is at least 40 times longer than that obtained with ADRIAMYCIN when tested in Swiss albino mice at equivalent doses.

41. A method for reducing tumor growth comprising administering the composition of claim 23.

42. A method for reducing tumor growth comprising administering the composition of claim 38 and 39.

43. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration comprising

(j) dissolving lipids comprising Distearoylphosphatidylcholine (DSPC) and cholesterol in a single solvent or in a mixture of solvents,

(k) removing said solvents before or after hydrating the lipids by addition of an aqueous hydration media to form liposomes in a liposomal composition, wherein said aqueous hydration media comprises ammonium sulfate and sucrose, and wherein the aqueous hydration media is added in quantities in the range of 10ml to 35ml per each mmole of DSPC;

(l) sizing the liposomes in the liposomal composition obtained at the end of step (b), to about 0.060μm – 0.16μm;

(m) removing extraliposomal ammonium sulfate from the liposomal composition that has undergone sizing at step (c), using a sucrose - histidine buffer solution comprising histidine hydrochloride and sucrose;

(n) dissolving doxorubicin hydrochloride in said sucrose - histidine buffer solution to obtain a solution of at least 25mM doxorubicin hydrochloride concentration;

(o) admixing doxorubicin hydrochloride solution obtained at step (e) and the liposomal composition obtained at the end of step (d) to obtain doxorubicin hydrochloride loaded liposomal composition;

(p) removing extraliposomal doxorubicin hydrochloride from the liposomal

composition by a process selected from the group consisting of tangential flow filtration, column chromatography and treatment with resins;

(q) making up the volume of the liposomal composition obtained at the end of step (g) with said sucrose - histidine buffer solution to obtain a liposomal composition of a desired concentration of doxorubicin hydrochloride;

(r) filtering aseptically, the liposomal composition through a sterile 0.2μ sterilising grade filter into a sterile container to obtain said liposomal doxorubicin composition.

44. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43 further comprising, filling the liposomal doxorubicin hydrochloride composition into sterile depyrogenated containers and sealing the container under cover of an inert gas.

45. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43 wherein the concentration of ammonium sulfate in the aqueous hydration media is not less than 125mM per liter.

46. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43, wherein, the molar ratio of sucrose to histidine hydrochloride in the sucrose – histidine buffer solution used in step d) is between 29:0.1 to 29:10.

47. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 46, wherein, the molar ratio of sucrose to histidine hydrochloride in the sucrose – histidine buffer solution is 29:1.

48. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43, wherein the doxorubicin hydrochloride concentration is from 1mM to 10mM.

49. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin

composition for parenteral administration as claimed in claim 48, wherein the doxorubicin hydrochloride concentration is about 3.45mM.

50. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43, wherein the molar ratio of distearoylphosphatidyl choline:cholesterol is from 1:0.6 to 1:0.8.

51. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 50, wherein the molar ratio of distearoylphosphatidyl choline:cholesterol is about 1:0.7

52. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43, wherein the molar ratio of doxorubicin hydrochloride:distearoylphosphatidyl choline is from 1: 2 to 1: 15.

53. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 52, wherein the molar ratio of doxorubicin hydrochloride:distearoylphosphatidyl choline is about 1:3.5

54. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in any claim 43, wherein the sucrose concentration is from 0.1 M to 0.5M.

55. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in any claim 54, wherein the sucrose concentration is from 0.25M to 0.3M.

56. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in any claim 55, wherein the concentration of histidine hydrochloride is from 1mM to 100mM.

57. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 55, wherein the

concentration of histidine hydrochloride is from 8mM to 12mM

58. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 56, wherein the concentration of histidine hydrochloride is about 10mM.

59. A process for manufacture of a long circulating non-pegylated liposomal doxorubicin composition for parenteral administration as claimed in claim 43, wherein half circulation time ($t_{1/2}$) in blood is at least 25 times longer than that obtained with ADRIAMYCIN when tested in Swiss albino mice at equivalent doses

60. A process for manufacture of long circulating non-pegylated sized liposomes comprising;
dissolving one or more phospholipids, and a sterol in a solvent or mixture of solvents;
removing said solvents before after hydrating the phospholipids by addition of a aqueous hydration media to form non-pegylated liposomes;
wherein the amount of the aqueous hydration media used is in the range of 10 to 35 ml for each mmole of phospholipid present in the lipid solution;
sizing the non-pegylated liposomes to about 0.06μm to 0.1μm to form a liposomal composition;
removing extra-liposomal hydration salt from the liposomal composition using sucrose-histidine buffer solution to form non-pegylated sized liposomes.